Supplemental Data

A30P α-Synuclein Adopts the Wild-type Fibril Structure, Despite Slower Fibrillation Kinetics *

Luisel R. Lemkau,[1] Gemma Comellas,[2] Kathryn D. Kloepper,[1,5] Wendy S. Woods,[3] Julia M. George[3] and Chad M. Rienstra[1,2,4]

[1]From the Department of Chemistry, [2]Center for Biophysics and Computational Biology, [3]Department of Cell and Developmental Biology and [4]Department of Biochemistry, University of Illinois, Urbana, IL 61801

[5]Department of Chemistry, Mercer University, Macon, GA, 31207

*Running title: *A30P α-synuclein fibrils adopt the wild-type structure*

To whom correspondence should be addressed: Chad M. Rienstra, Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA Tel.: (217) 244-4655; Fax: (217) 244-3186; E-mail: rienstra@scs.illinois.edu.

Solution NMR spectroscopy. Monomeric AS samples were prepared for solution NMR experiments as 400 μL solutions containing 0.33 mM ^{15}N labeled monomeric AS in 50 mM phosphate buffer (pH 7.5, 0.02% NaN3 w/v), 10% D_2O and 1 mM DSS. A two-dimensional (2D) BEST ^{1}H-^{15}N heteronuclear single-quantum correlation (HSQC) spectrum (1,2) was acquired of WT and A30P AS monomeric samples, Figure SI1. Solution NMR experiments were conducted on a Varian INOVA 14 Tesla (600 MHz, ^{1}H frequency) spectrometer using a triple resonance (^{1}H-^{13}C-^{15}N) triaxial gradient probe, utilizing VNMRJ version 2.1B software with BioPack. All solution NMR spectra were acquired at 0 ^{o}C. Chemical shift assignments labeled in Figure S1 are based on the published assignments that are deposited on the Biological Magnetic Resonance data Bank (BMRB) with the accession numbers #16300 (3,4) and #16546 (5,6) for the WT and A30P, respectively. The amide proton chemical shift of A30 is reported to be 8.26 ppm and 123.54 ppm of the amide nitrogen for the WT monomer. We did not see a correlation at these frequencies in either spectrum. However, we found that the chemical shifts of A29 and G31-T33 had been perturbed as previously described upon mutation.

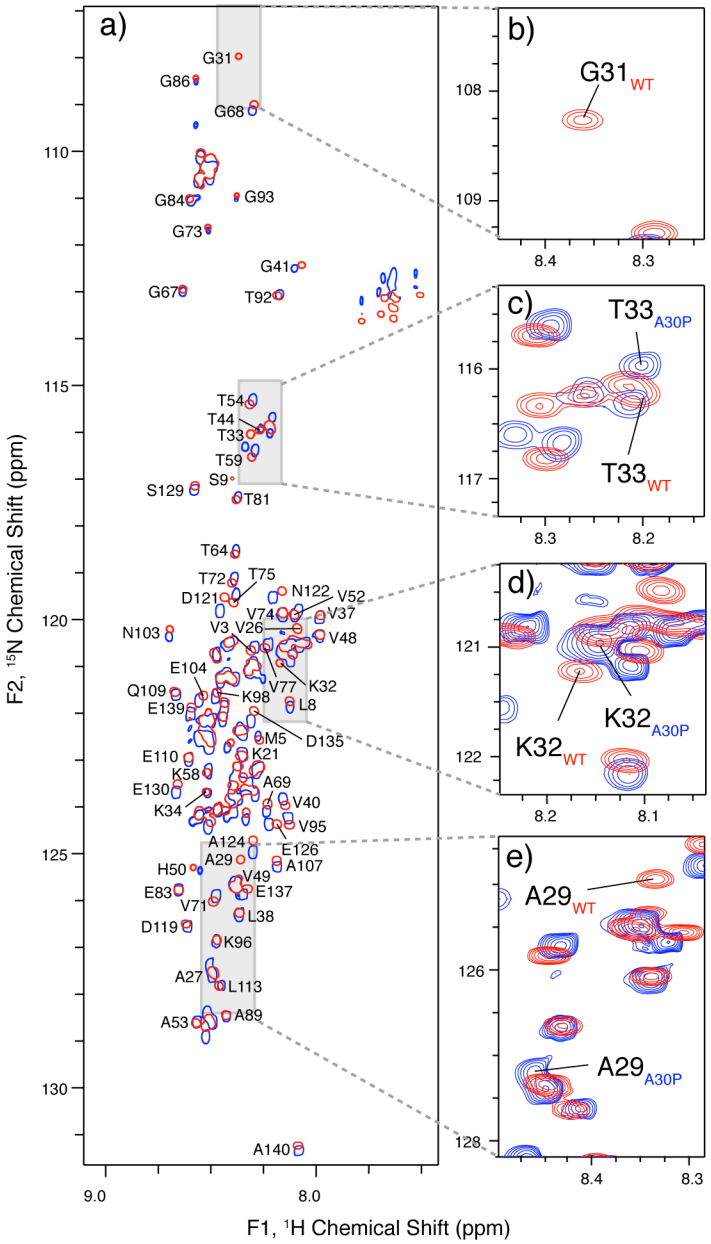

FIGURE S1. Solution NMR demonstrates the A30P mutation was present before fibrillation. (a) Overlaid ^{1}H-^{15}N HSQC spectra of A30P (blue) and WT (red) purified AS monomer. Expansions of residues near mutation site: (b) G31, (c) T33, (d) K32 and (e) A29. Chemical shift assignments based on the deposited solution assignments from the BMRB for WT # 16300 (4) and A30P #16546 (6).

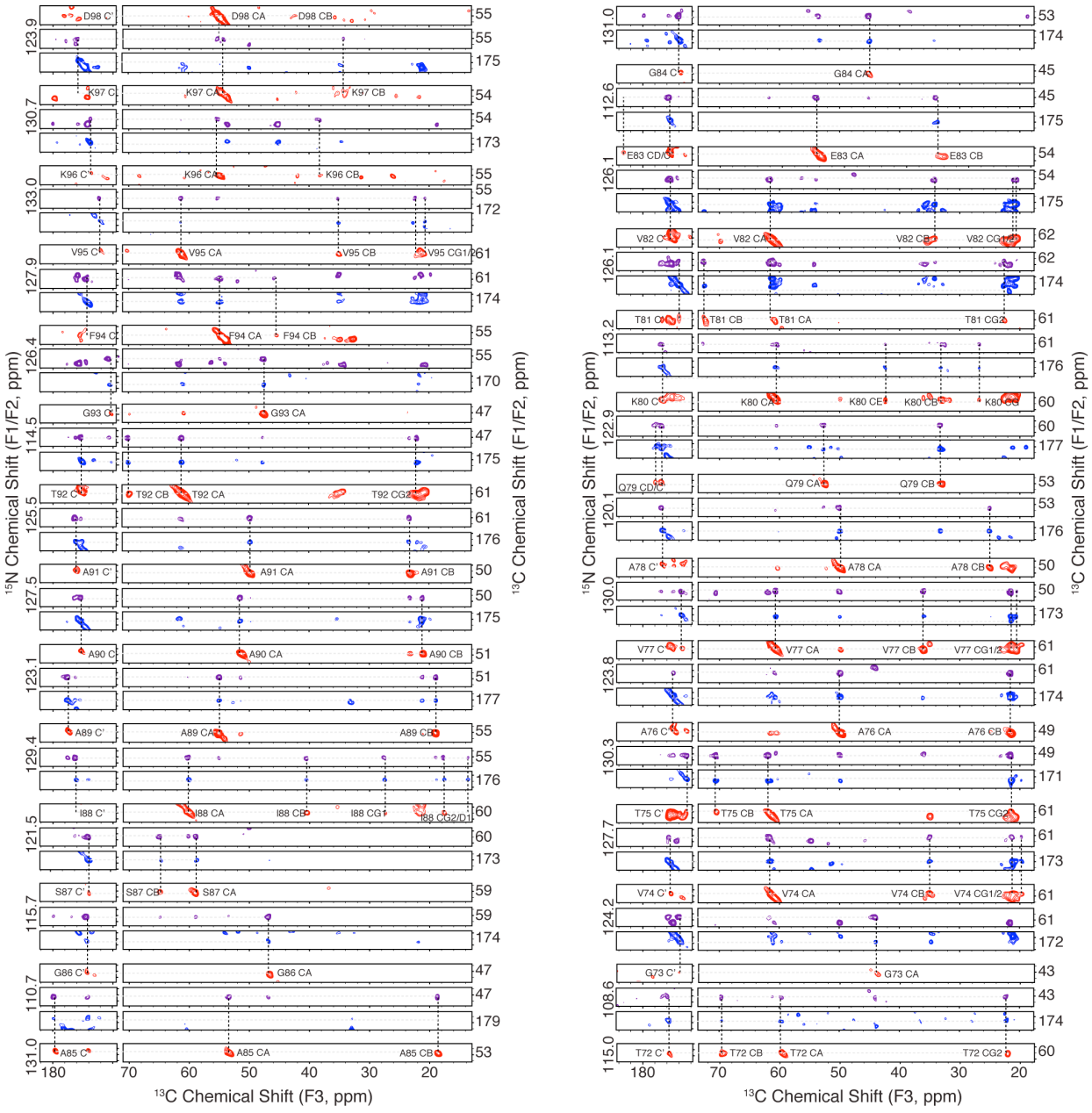

FIGURE S2. Backbone walk scheme using NCACX (red) with 50 ms DARR, CAN(CO)CX (purple) with 50 ms DARR and NCOCX (blue) with 100 ms DARR spectra of A30P AS fibrils; residues D98 to T72. Spectra were acquired at a VT temperature of 10 ^{o}C (sample temperature ~13 ^{o}C) and 13.3 kHz MAS. Acquisition and processing details are described in the Materials and Methods section.

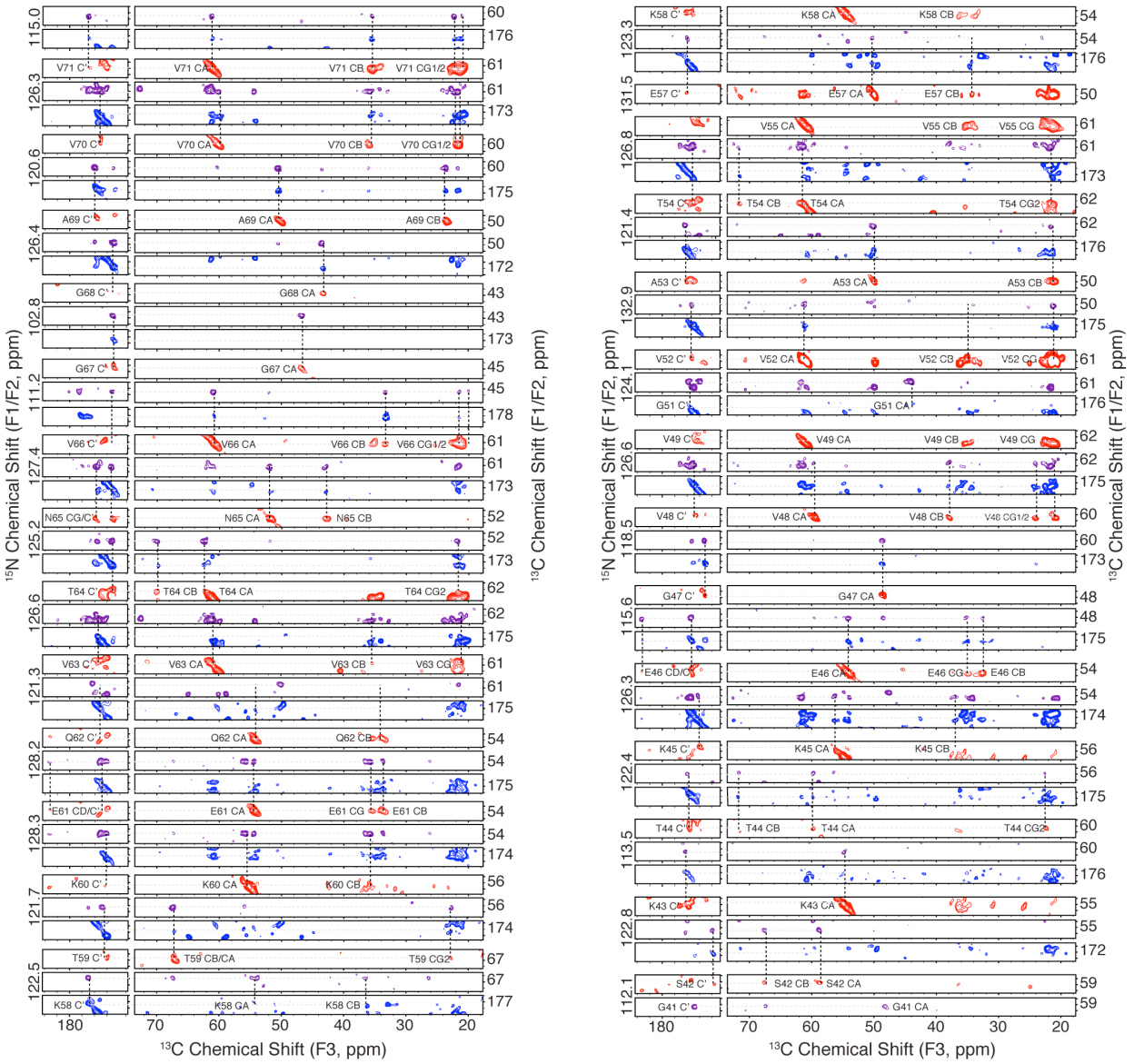

FIGURE S3. Backbone walk scheme using NCACX (red) with 50 ms DARR, CAN(CO)CX (purple) with 50 ms DARR and NCOCX (blue) with 100 ms DARR spectra of A30P AS fibrils; residues V71 to G41. Spectra were acquired at at a VT temperature of 10 ^{o}C (sample temperature ~13 ^{o}C) and 13.3 kHz MAS. Acquisition and processing details are described in the Materials and Methods section.

TABLE S1. Description of the 2D and 3D experiments acquired in order to obtain the *de novo* ^{13}C, ^{15}N chemical shift assignments for U-^{13}C, ^{15}N A30P AS fibrils. Chemical Shifts were deposited to the BMRB, entry #17214. The total amount of protein was calculated based on the intensity of a ^{13}C 1D DP experiment versus that of adamantane (the mass for adamantane was determined before packing the sample in the rotor).

Experiments	Dimensions acquired	Mixing time and sequence	Acquisition Time (hrs)	Amount of sample (mg)
CC	2D	50 ms DARR	11	~20
CC	2D	250 ms DARR	9	~20
CC	2D	500 ms DARR	58	~20
N(CA)CX	2D	50 ms DARR	19	~10
N(CA)CX	2D	200 ms DARR	10	~20
NCACX	3D	50 ms DARR	110	~10
NCACX	3D	200 ms DARR	100	~20
N(CO)CX	2D	50 ms DARR	16	~10
N(CO)CX	2D	250 ms DARR	48	~20
NCOCX	3D	100 ms DARR	120	~10
NCOCX	3D	250 ms DARR	180	~20
CA(NCO)CX	2D	50 ms DARR	14	~20
CANCO	3D	----	42	~20
CAN(CO)CX	3D	50 ms DARR	135	~20

TABLE S2. ^{13}C and ^{15}N chemical shift assignments of A30P AS fibrils (with 36% water by mass) using only uniformly labeled samples. Chemical shifts were deposited to the BMRB, entry #17214. WT-AS fibril assignments were made with data acquired on samples with selected ^{13}C labeling indicated by an asterisk (*) and assignments not possible with either selected or uniform labeling are indicated in **bold**. Assignments in *italics* are tentative for A30P fibrils.

Residue	^{15}N	$^{13}C'$	^{13}CA	^{13}CB	^{13}CG	^{13}CD	^{13}CE	^{13}CZ	^{15}ND	^{15}NE	^{15}NZ
Y39	---	---	54.3*	35.4*	---	---	---	---			
V40	126.4*	**176.4**	61.1*	35.5*							
G41	115.2*	174.5*	48.4*								
S42	112.1*	171.5*	58.6*	67.4*							
K43	122.8*	175.7*	54.7*	38.0*	25.8*	31.1*	41.9*				---
T44	113.5	175.3	59.6	71.5	22.3						
K45	122.5	173.8	56.2	37.0	27.5	30.8	42.3				---
E46	126.4	175.0	54.0	32.5	35.1	182.9					
G47	115.6	172.7	48.4								
V48	118.5	174.6	59.6	37.7	24.0/20.8						
V49	126.6	174.7	61.6	34.0	23.0/21.2						
G51	---	**175.8**	44.9*								
V52	124.1	174.7	61.3	34.0	21.2/20.7						
A53	132.9	175.8	50.0	21.2							
T54	121. 4	173.4	61.6	71.5	22.0						
V55	126.8	---	60.8	34.2	23.2/22.8						
E57	---	**175.5**	**50.2**	**34.1**	**35.7**	---					
K58	**123.3**	176.5*	54.0*	36.3*	25.9*	32.8*	42.1*				---
T59	122.5*	174.3*	67.2	67.3	22.7						
K60	121.7*	**173.7**	55.7*	**35.6**	**26.5**	**32.2**	**42.5**				---
E61	**128.3**	**174.8**	**54.2**	**33.6**	**36.5**	**183.0**					
Q62	---	175.1*	**49.3**	**34.7**	**35.6**	---				---	
V63	122.9	175.2	61.1	35.4	21.3/20.4						
T64	126.6	172.7	62.2	69.8	21.8						
N65	125.2	172.6	51.8	42.9	175.2				115.0		
V66	127.4	177.8	60.7	33.2	**21.2/20.0**						
G67	111.2	172.7	46.5								
G68	102.8	172.4	43.3								
A69	126.4	175.4	50.3	23.4							
V70	120.6	174.6	60.1	35.7	21.5						
V71	126.3	176.5	61.0	35.2	22.0/20.9						
T72	115.0	175.3	59.5	69.6	22.1						
G73	108.6	173.4	43.9								
V74	124.2	175.1	61.4	34.9	21.0/19.6						
T75	127.7	172.1	61.7	70.5	21.4						
A76	130.3	174.4	49.7	21.4							
V77	123.8	172.9	60.5	35.9	21.3/20.4						
A78	130.0	176.4	49.9	25.0							
Q79	120.1	176.6	52. 5	33.1	33.3	177.6				110.8	
K80	122.9	176.1	60.3	32.5	26.6	31.5	42.3				---
T81	113.2	173.7	60.9	72.4	22.5						
V82	126.1	174.9	61.5	34.1	21.5/20.7						
E83	126.1	175.2	53.8	33.8	35.8	182.9					
G84	112.6	173.6	45.0								
A85	131.0	179.3	53.3	18.6							
G86	110.7	173.8	46.7								
S87	115.7	173.5	58.8	64.9							
I88	121.5	175.7	60.1	40.5	27.4/17.6	13.5					
A89	129.4	176.9	54.9	18.8							

A90	123.1	174.8	51.3	21.0						
A91	127.5	175.6	49.6	23.1						
T92	125.5	174.7	61.0	70.0	21.8					
G93	114.5	170.1	47.4							
F94	126.4	173.3	54.6	45.4	138.4	132.4/132.2	131.0/130.7	129.4		
V95	127.9	171.5	61.1	34.9	22.1/20.7					
K96	133.1	173.2	54.9	**38.0**	**25.9**	**31.0**	**42.7**			---
K97	130.7*	**175.1**	54.1*	**34.3**	**25.6**	**---**	**42.2**			---
D98	**124.0**	**177.1**	**55.1**	**42.6**						

REFERENCES

1. Schanda, P., Van Melckebeke, H., and Brutscher, B. (2006) *J. Am. Chem. Soc.* **128**, 9042-9043
2. Schanda, P., Lescop, E., Falge, M., Sounier, R., Boisbouvier, J., and Brutscher, B. (2007) *J. Biomol. NMR* **38**, 47-55
3. Eliezer, D., Kutluay, E., Bussell, R., and Browne, G. (2001) *J. Mol. Biol.* **307**, 1061-1073
4. Rao, J. N., Kim, Y. E., Park, L. S., and Ulmer, T. S. (2009) *J. Mol. Biol.* **390**, 516-529
5. Bussell, R., and Eliezer, D. (2001) *J. Biol. Chem.* **276**, 45996-46003
6. Bodner, C. R., Maltsev, A. S., Dobson, C. M., and Bax, A. (2010) *Biochemistry* **49**, 862-871